

690    ***In vivo calcineurin activity assay***

691    PxIxIT peptides were fused to eGFP in pEGFPc1 vector. HEK293T cells were transfected with pEGFPc1  
 692    clones, pNFAT-Luc and CMV-Renilla in a 6-well plate format. 18 hours post transfection, 1 $\mu$ M FK506  
 693    or DMSO (vehicle) was added to the media as needed. 36 hours post transfection, cells were  
 694    treated with 1mM Ionomycin and 1mM phorbol 12,13 di-butrate to activate calcineurin and AP-1  
 695    (via PKC) respectively. 6 hours after pathway activation, cells were collected, washed in PBS and  
 696    re-suspended in DMEM media. 80% of the cells were used to measure luciferase activity and  
 697    renilla using the Dual-Glo assay system (Promega) with 3 technical replicates. The remaining cells  
 698    were frozen and stored at -80°C. Cell lysates were prepared in RIPA buffer. 15-20  $\mu$ g of lysate was  
 699    analyzed by Western for expression of GFP. GFP signal was normalized to either actin or tubulin.  
 700    Luciferase activity (normalized to renilla expression) was further normalized to eGFP expression.  
 701    Data represent at least 3 experimental replicates.

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711    **References**

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